Clone The mutants in pTTQ19

Plan: Delete 5'→3' exo activity of Tne Pol mutants (F→Y) and 3'-5'/F-Y

Digest pTTQ19 with Sph + SmaI. Clone Tne mutants at sphI and SmaI site. The digest with sphI/SmaI (HindIII) will put Tne pol in frame and thus, there was need for any other manipulation.

pTTQ19 : 5 µl (0.5 µg)
TE 20 µl
React 4 3 µl
sphI/Sma 1 µl/1 µl

30'/37°C. → freeze until other two are ready

pUC19FY : 60 µl DNA
6 µl React 2
2 µl HindIII

pUC35FY (#2) : 60 µl
6 µl React 2
2 µl HindIII

After 30' at 37°C add 5 µl 1mM dNTP mix + 1 µl (5U) Klenow — Incubate for 5' on ice. Add EDTA 2 µl ?mM → EtOH ppt → see next page.

-10 RBS
AATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCG
met asn ser arg gly ser val asp leu gln pro ser leu ala leu ala
ATG AAT TCC CGG GGA TCC GTC GAC CTG CAG CCA AGC TTG GCA CTG GCC
EcoRI SmaI BamHI SalI PstI HindIII
pTTQ8

-10 RBS
AATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCG
met ser leu ala ala gly arg arg ile pro gly asn ser leu ala
ATG AGC TTG GCT GCA GGT CGA CGG ATC CCC GGG AAT TCA CTG GCC
PstI SalI BamHI SmaI EcoRI
pTTQ9

-10 RBS
AATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCG
met asn ser ser ser val pro gly asp pro leu glu ser thr cys arg his ala ser leu ala leu ala
ATG AAT TCG AGC TCG GTA CCC GGG GAT CCT CTA GAG TCG ACC TGC AGG CAT GCA AGC TTG GCA CTG GCC
EcoRI SstI KpnI SmaI BamHI XbaI SalI PstI SphI HindIII
pTTQ18 ✓

-10 RBS
AATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCG
met ser leu his ala cys arg ser thr leu glu asp pro arg val pro ser ser asn ser leu ala
ATG AGC TTG CAT GCC TGC AGG TCG ACT CTA GAG GAT CCC CGG GTA CCG AGC TCG AAT TCA CTG GCC
SphI PstI SalI XbaI BamHI SmaI KpnI SstI EcoRI
← pTTQ19

-10 RBS
AATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCG
met asn leu ile thr asn ser ser ser val pro gly asp pro leu glu ser thr cys arg his ala ser leu
ATG AAT TTG ATT ACG AAT TCG AGC TCG GTA CCC GGG GAT CCT CTA GAG TCG ACC TGC AGG CAT GCA AGC TTG
Eco RI SstI KpnI SmaI BamHI XbaI SalI PstI SphI HindIII
pTTQ181

-10 RBS
TATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCT
met thr met ile thr asn ser ser ser val pro gly asp pro leu glu ser thr cys arg his ala ser leu
ATG ACC ATG ATT ACG AAT TCG AGC TCG GTA CCC GGG GAT CCT CTA GAG TCG ACC TGC AGG CAT GCA AGC TTG
EcoRI SstI KpnI SmaI BamHI XbaI SalI/AccI PstI SphI HindIII
pUC18

.otide sequences of the promoter and polylinker regions of the pTTQ vectors and pUC18. Sequence extending from the −35 region of the *lac* or *tac* promoter to the dist: polylinker is given for pTTQ8, 9, 18, 19 and 181. The comparable region of pUC18 is also shown. Unique cloning sites in the polylinker, the −35 and −10 regions of th d the RBS are shown.

[signature] 8/1/95 Recorded By [signature] 8/9/95

[redacted]

40 Project No._____ Book No._____ TITLE Tne clones in pTTQ19 (4'-5'-exo)

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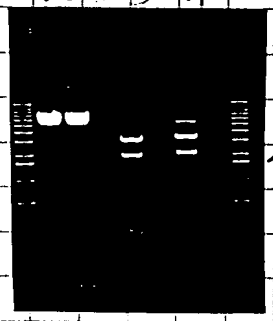
pUC Tne FY HindIII → blunt ended fragment → dissolve in 17µl T

pUC Tne 35FY HindIII → blunt end fragment → dissolve in 17µl T

Sph I digestion

	FY	35 FY
DNA	17µl	17µl
React 6	2µl	2µl
Sph I	1µl	1µl

30 min / 37°c → Run gel.



#1 & #2 → pTTQ19 sph / sma

#3 → pUC Tne FY

#4 → pUC Tne 35 FY

purify Vector & insert as a mixture by gene clean in H_2O.

ⓐ pTTQ19 + 2.0 Kb (Tne FY)

ⓑ pTTQ19 + 2.0 Kb (Tne 35FY)

ligation

15µl DNA mix
4µl 5 x buffer
1µl SU ligase

Ligate for 15 min at room temp.

Transform DH10B. Plate 10% & 90% culture – 30°C / ON.

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Witnessed & Understood by me,	Date	Invented by	Date
	8/7/95	R c rded by	7/20/95

Tne clones in pTTQ19. (Δ 5'-exo)

Proj ct No.
B ok N .

Pag No.

Result of transformation:

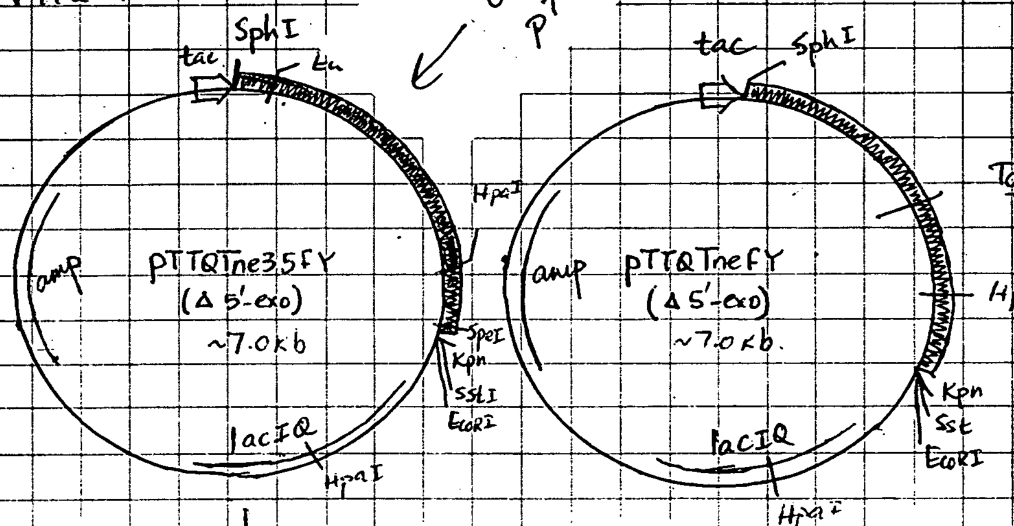
pTTQ Tne 35/FY :	10%	105
	90%	TMTC
pTTQ Tne FY	10%	115
	90%	TMTC.

Left the plates in the bench over the weekend.

Inoculate 6 clones from " for mini prep (2 mL EG/Amp).
Overnight growth at 30°C.

Standard mini prep! was dissolve in 150µl TE.
Digest clones with SstI. The SstI will be coming from pTTQ19.

Listed as pTTQTne535FY in App.



To be made later

Please see Mary Longo's note book # 57 (LTI Book # 3959, p183).

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[signature]	8/7/95	Recorded by Deb K [signature]	7/21/95